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Inorganic Ring Systems of Physiological Importance. Part II[+]. Substituent Effects in the Aziridinolysis of $(NPCl_2)_3$

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The aziridinolysis of $(NPCl_2)_n(NPAmCl)_{3-n}$ (n = 1, 2; Am = pyrrolidino, piperidino, morpholino) in diethyl ether is described. In a series of comparable reactions it is found that the replacement of chlorine atoms takes place at PCl_2 rather than at PAmCl centres. Thus, the presence of the secondary amino ligands causes a retardation of the aminolysis of the PAmCl centres by the weak nucleophile aziridine (ethylene imine, $HN(CH_2)_2$). Concerning the reactivity of the PAmCl centres the following sequence is observed:*

PPyrCl > PPipCl > PMorphCl

Apart from steric effects this sequence can be related to differences in the electron-donating ability of the amino groups, giving rise to changes in the leaving group capacity of the chlorine atoms. Moreover, the reactivity of the PAmCl centres in $NPCl_2(NPAmCl)_2$ is related to the mutual position of the amino substituents.

Introduction

Aziridino derivatives are considered to be potential mutagenic and cytotoxic agents. Closely related herewith is their application as anticancer drugs. Some aziridino derivatives of $(NPCl_2)_3$ (I) are known to be effective against animal tumors [1–5]. Recently we found that this also applies to aziridino derivatives of the sulphur-containing ring system $(NPCl_2)_2NSOCl$ (II) [6–9] (Fig. 1). Series of compounds containing both aziridino and pyrrolidino or morpholino substituents have been summarized earlier by Kropacheva *et al.* [3, 10, 11], while Ottmann as well as Kobayashi and their coworkers prepared a number of mixed dimethylamino–aziridino derivatives [12–14].

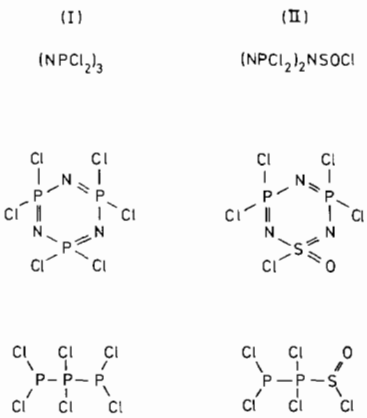

Fig. 1. Structures of $(NPCl_2)_3$ (I) and $(NPCl_2)_2NSOCl$ (II); in the side-views the nitrogen atoms are omitted.

For a comparison of the analogues of (I) and (II) with respect to their behaviour as anti-tumor agents, we started the synthesis of a series of mixed aziridino–cyclic secondary amino (*viz.* Pyr, Pip, Morph) derivatives of (I).

This investigation concerns the reactions of $(NPCl_2)_n(NPAmCl)_{3-n}$ (n = 1, 2) with aziridine in diethyl ether. Ottmann *et al.* [12] and Kobayashi *et al.* [14] stated that the remaining chlorine atoms in bis-, tris- and tetrakis (dimethyl-amino) derivatives of (I) could be replaced only using rather drastic reaction conditions. However, they did not investigate this unreactivity in detail. A similar behaviour was observed in our experiments, as will be discussed later on.

In order to compare the reactivity of the various precursors the successive reaction conditions (temperature, solvent, concentration and molar ratio) were standardized. The low nucleophilicity of aziridine enables one to detect even small variations in reactivity which otherwise would be overlooked.

Evidence for the difference in reactivity was provided by the analysis of crude reaction mixtures using ^{31}P NMR and mass spectrometry. The definitive characterization of most of the products was carried out after isolation by recrystallization or HPLC techniques.

[+]Part I: see ref [6].

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*In this paper the following abbreviations are used: Am (amino), Pyr (pyrrolidino), Pip (piperidino), Morph (morpholino), Az (aziridino).

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Experimental

All experiments were carried out under dry nitrogen. Pyrrolidine, piperidine, morpholine and aziridine were purified before use by distillation over KOH pellets. Solvents were purified and dried by conventional methods. Elemental analyses were carried out at the Microanalytical Department of this University under the supervision of Mr. A. F. Hamminga. Mass spectra were recorded on an AEI M.S. 9 mass spectrometer operating at 70 eV, using an accelerating voltage of 8 kV. Samples were introduced directly by a conventional inlet system at about 100 °C (Mr. A. Kiewiet, Department of Organic Chemistry, this University). ^{31}P NMR spectra were taken with a Varian XL-100 FT spectrometer in 5 mm tubes at 37 °C at 40.5 MHz. Chemical shifts in ppm were determined relative to the external standard of 85% H_3PO_4. The deuterium resonance of the solvent ($CDCl_3$) was used for field-frequency lock (Mr. C. Kruk, University of Amsterdam). ^1H NMR spectra were recorded at 35 °C on a Varian A-60 spectrometer using TMS as the internal reference. Purification by HPLC was carried out using a Spectra-Physics 740 B pump and a L.D.C. refractometer (model 1107). Separations were performed on Lichrosorb Si 60/10 columns with n-hexane/diethyl ether mixtures as eluents (Mr. H. P. Velvis, Department of Inorganic Chemistry, this University).

I. Preparation of the Starting Materials $(NPCl_2)_2$-NPAmCl and $NPCl_2(NPAmCl)_2$

General procedure

To a vigorously stirred solution of 5 mmol of (I) in 40 ml of solvent, cooled at −17 °C, was added dropwise a solution of the appropriate amount of amine in 40 ml of solvent. The reaction mixture was allowed to warm up slowly to room temperature. Removal of the precipitated amine hydrochloride by filtration and evaporation of the filtrate to dryness gave the crude product. Purification was performed by HPLC and recrystallization from dry n-hexane or diethyl ether. Data on the syntheses are given in Table I.

II. Reactions of $(NPCl_2)_2NPAmCl$ with Aziridine in Diethyl Ether

Two different procedures were applied.

A (Reactions 1–3)

A solution of aziridine in diethyl ether was added dropwise to a stirred ethereal solution of $(NPCl_2)_2$-NPAmCl, which was cooled at −75 °C (molar ratio 20:1). The reaction mixture was allowed to warm up slowly to room temperature and stirring was continued for two days at that temperature.

TABLE I. Preparative Data on the Compounds $(NPCl_2)_n(NPAmCl)_{3-n}$.

Compound	Solvent	Amount of amine (mmol)	Molar ratio (I)/amine	Purification method	Yield (%)	M.p. (°C)[a]	
$(NPCl_2)_2$NPPyrCl III	Et_2O	10	1:2	HPLC recrystd. from n-C_6H_{14}	22	66.5–67.0	(65.5–66.5)
$(NPCl_2)_2$NPPipCl IV	n-C_6H_{14}	10	1:2	recrystd. from n-C_6H_{14}	46	66.5–68.0	(68)
$(NPCl_2)_2$NPMorphCl V	Et_2O	10	1:2	"	41	91.0–92.0	(92.5–93.5)
trans-$NPCl_2$(NPPyrCl)$_2$ VI	n-C_6H_{14}	20	1:4	"	80	55.0–56.0	(56–56.5)
trans-$NPCl_2$(NPPipCl)$_2$ VII	n-C_6H_{14}	20	1:4	"	62	104–105	(104–105)
trans-$NPCl_2$(NPMorphCl)$_2$ VIII	n-C_6H_{14}	20	1:4	"	50	103–104	(106–108)
cis-$NPCl_2$(NPPyrCl)$_2$ IX	MeCN	20	1:4	HPLC recrystd. from n-C_6H_{14}	48	96.0–97.0	(94.5–95.5)
cis-$NPCl_2$(NPPipCl)$_2$ X	MeCN	20	1:4	"	30	124–125	(129)
cis-$NPCl_2$(NPMorphCl)$_2$ XI	MeCN	20	1:4	recrystd. from Et_2O	30	192–193	(202–203)

[a]Values in parentheses are taken from reference [15].

B (Reactions 4, 5)

Procedure B differs from A in cooling the solution of $(NPCl_2)_2NPAmCl$ at 0 °C and using a molar ratio of 30:1. Furthermore, after standing overnight, the reaction mixture was refluxed at 35–40 °C during five hours.

All reaction mixtures were worked up by removal of the aziridine hydrochloride salt by filtration. The residue was washed with diethyl ether and the combined filtrate and extracts were evaporated to dryness. In general, two main products appeared to be present (from mass and ^{31}P NMR spectra), *i.e.* $(NPAz_2)_2NPAmCl$ and $(NPAz_2)_2NPAmAz$. Relative amounts were calculated from the ^{31}P NMR spectra by comparison of peak heights.

After recrystallization of the crude products from diethyl ether reactions 2, 3 and 4 yielded $(NPAz_2)_2$-NPPipCl (XIV), $(NPAz_2)_2NPMorphCl$ (XVI) and $(NPAz_2)_2NPPyrAz$ (XIII), respectively. The 'complementary' compounds, *viz.* $(NPAz_2)_2NPPipAz$ (XV) $(NPAz_2)_2NPMorphAz$ (XVII) and $(NPAz_2)_2NPPyrCl$ (XII) were obtained in a pure state as described below (Part IV). Data are given in Table II.

III. Reactions of $NPCl_2(NPAmCl)_2$ with Aziridine (molar ratio 1:16) in Diethyl Ether

Reactions 6–11

Use was made of procedure A (Part II). Except for reaction 6 only a single product was detected in the crude reaction mixtures. By recrystallization from a diethyl ether/n-pentane mixture pure products with general formula $NPAz_2(NPAmCl)_2$ were isolated. No attempts were made for isolating the products of reaction 6. *Trans*-$NPAz_2(NPPyrAz)_2$ (XVIII) and *trans*-$NPAz_2(NPPyrCl)_2$ (XX) were prepared by different methods as described below (Part IV). Table II gives detailed data.

IV. Preparation of Additional Compounds

Preparation of (XII)

To a stirred solution of 1.40 mmol of (III) and 6.80 mmol of triethylamine in 20 ml of benzene, cooled at 5 °C, a solution of 5.60 mmol of aziridine in 20 ml of benzene was added dropwise. After warming up to room temperature, stirring of the reaction mixture was continued overnight. The temperature was then raised to 35 °C for one day. The hydrochloride salt was filtered off and evaporation of the filtrate to dryness yielded a white solid. After several recrystallizations 0.35 mmol (25%) of pure (XII) was obtained.

Preparation of (XVIII)

To a stirred solution of 1.95 mmol of (VI) in 25 ml of benzene, cooled at 5 °C, was added dropwise a solution of 31.4 mmol of aziridine in 20 ml of

TABLE II. Data on the Reactions 1–11.

Reaction	Precursor	Mmol	Et_2O (ml)	Aziridine (mmol)	Et_2O (ml)	Molar ratio reactants	Temp. range (°C)	Products		Relative amounts (%)	Yield (%)
1	III	1.05	20	20.9	10	1:20	−75/25	$(NPAz_2)_2NPPyrCl$	XII	38	–
								$(NPAz_2)_2NPPyrAz$	XIII	62	–
2	IV	1.84	30	36.8	20	1:20	−75/25	$(NPAz_2)_2NPPipCl$	XIV	67	39
								$(NPAz_2)_2NPPipAz$	XV	33	–
3	V	1.00	20	20.1	10	1:20	−75/25	$(NPAz_2)_2NPMorphCl$	XVI	100	70
4	III	1.84	40	55.3	30	1:30	0/35	$(NPAz_2)_2NPPyrAz$	XIII	100	56
5	V	1.91	40	57.2	30	1:30	0/35	$(NPAz_2)_2NPMorphCl$	XVI	86	43
								$(NPAz_2)_2NPMorphAz$	XVII	14	–
6	VI	2.34	40	37.5	30	1:16	−75/25	*trans*-$NPAz_2(NPPyrAz)_2$	XVIII	not det.	–
								cis-$(NPAz_2)(NPPyrAz)(NPPyrCl)$	XIX	,,	–
								trans-$NPAz_2(NPPyrCl)_2$	XX	,,	–
7	VII	1.68	30	27.0	25	1:16	−75/25	*trans*-$NPAz_2(NPPipCl)_2$	XXI	100	60
8	VIII	2.23	40	35.7	30	1:16	−75/25	*trans*-$NPAz_2(NPMorphCl)_2$	XXII	100	55
9	IX	1.20	25	19.2	20	1:16	−75/25	*cis*-$NPAz_2(NPPyrCl)_2$	XXIII	100	71
10	X	0.95	20	15.1	15	1:16	−75/25	*cis*-$NPAz_2(NPPipCl)_2$	XXIV	100	72
11	XI	0.65	60	10.4	15	1:16	−75/25	*cis*-$NPAz_2(NPMorphCl)_2$	XXV	100	58

benzene. A procedure similar to that used for the preparation of (XII) yielded 0.78 mmol (40%) of (XVIII).

Preparation of (XX)

To a stirred solution of 1.88 mmol of (VI) in 30 ml of diethyl ether, cooled at −75 °C, was added dropwise a solution of 7.89 mmol of aziridine in 20 ml of diethyl ether. The reaction mixture was allowed to warm up to room temperature.

Stirring was continued for an additional period of three days. The hydrochloride salt was filtered off and the filtrate was evaporated to dryness. The crude product was recrystallized from petroleum ether and yielded 0.56 mmol (30%) of (XX).

Preparation of (XV) and (XVII)

$N_3P_3Az_5Cl$ was prepared as starting material according to the method of Kobayashi *et al.* [14]. To a stirred solution of 2.60 mmol of this precursor in 15 ml of benzene, cooled at 5 °C, was added dropwise a solution of piperidine (or morpholine) in 15 ml of benzene. After warming up slowly to room temperature, the reaction mixture was kept at 35–40 °C for three days. Then the hydrochloride salt was filtered off and the filtrate evaporated to dryness. By recrystallization from diethyl ether 1.59 mmol (61%) of (XV) and 2.03 mmol (73%) of (XVII) were obtained.

V. Preparation of $(NPPip_2)_2NPPipCl$ (XXVI) and $(NPMorph_2)_2NPMorphCl$ (XXVII)

Both compounds were prepared according to the general procedure given in I, using acetonitrile as a solvent and molar ratios (I):amine of 1:12 and 1:20.4 respectively. The compounds were obtained as white crystalline solids after recrystallization from diethyl ether, in yields of 45 and 60% respectively.

Preparative and analytical data on the compounds XII–XXVII are given in Table III.

Discussion

In general, substitution reactions of (I) with secondary amines lead to non-geminally substituted reaction products. Once an amino substituent is introduced, the susceptibility of PAmCl centres towards nucleophilic attack is reduced considerably. Aziridine however is the only secondary amine that tends to react with (I) along a geminal route [14, 16]. However, these papers give an over-simplified picture, as analysis of crude reaction mixtures by ^{31}P NMR shows high yields of non-geminal isomers [17], as well as geminal products.

In our experiments the excessive amounts of aziridine used would be expected to lead to completely aminolysed products. However, in almost all reaction mixtures considerable amounts of incompletely aminolysed products are present (see Table II). Obviously, aziridine prefers to attack PCl_2 centres, whereas the PAmCl centres remain relatively unaffected. This reaction pattern also points (at least partly) to non-geminal behaviour of aziridine in substitution reactions.

Considering in detail the reactivity of the PAmCl centres, from reactions 1–5 a shift towards a decreasing reactivity can be observed in the order:

TABLE III. Characterization of Compounds.

Compound	M.p(°C)[a]	Elemental Analyses (%)[b]			
		C	H	N	Cl
XII	139–140	34.73(34.84)	5.79(5.85)	26.80(27.08)	not det.
XIII	103–104 (104.5–105.5)	40.11 (40.48)	6.81 (6.79)	30.05 (30.35)	
XIV	141–142.5	37.27 (36.93)	6.27 (6.19)	26.23 (26.50)	not det.
XV	76.5–78.0	42.10 (41.96)	7.07 (7.04)	28.89 (29.36)	
XVI	161–162 (171–173)	34.24 (33.93)	5.73 (5.70)	26.15 (26.38)	not det.
XVII	115–116 (120.5–121.5)	38.91 (38.98)	6.57 (6.54)	29.22 (29.22)	
XVIII	99–100 (96–98)	43.36 (43.34)	7.24 (7.27)	28.13 (28.43)	
XX	129–130 (133–135)	33.40 (33.50)	5.72 (5.62)	22.51 (22.79)	16.69 (16.48)
XXI	137–138	37.24 (36.69)	6.33 (6.16)	21.41 (21.40)	15.32 (15.47)
XXII	172–174	31.23 (31.17)	5.23 (5.19)	21.08 (21.21)	15.35 (15.37)
XXIII	121–122 (117–119)	33.69 (33.50)	5.62 (5.62)	22.88 (22.79)	16.42 (16.48)
XXIV	126–127	36.93 (36.69)	6.36 (6.16)	21.23 (21.40)	15.16 (15.47)
XXV	158–159.5	31.35 (31.17)	5.22 (5.19)	21.19 (21.21)	15.37 (15.37)
XXVI	116–116.5	51.04 (50.80)	8.46 (8.53)	18.97 (18.96)	6.01 (6.00)
XXVII	166–174	40.38 (39.97)	6.92 (6.71)	18.62 (18.65)	5.90 (5.90)

[a]Values in parentheses are taken from the literature [3, 10]. [b]Calculated values in parentheses.

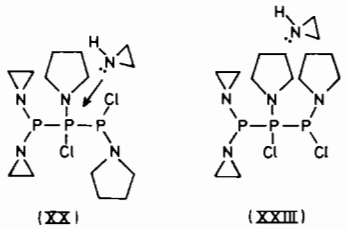
PPyrCl > PPipCl > PMorphCl

Using the *trans*-diamino derivatives as precursors (reactions 6–8), again the higher reactivity of the pyrrolidino derivative towards aziridinolysis can be distinguished from the two other ones. This difference disappears in the case of the *cis*-isomers (reactions 9–11) by the complete unreactivity of all PAmCl centres under the reaction conditions used.

Our results can be partly ascribed to steric effects. The unreactivity of the *cis*-tetrakis(amino) products, formed in reactions 9–11, can be explained by the mutual arrangement of the amino substituents. This is underlined by the different courses of reactions 6 and 9. Apparently, the presence of two pyrrolidino groups and an aziridino group on the same side of the P–N ring in case of the *cis*-isomer (XXIII) leads to an effective shielding against the attack by aziridine (Fig. 2).

This holds in general for all *cis*-tetrakis(amino derivatives as, for example, we were only able to convert *cis*-$NPAm_2(NPAmCl)_2$ into $(NPAm_2)_3$ (Am = Pyr, Pip, Morph) using very drastic reaction conditions, whereas the *trans*-isomers react smoothly [18]. It should be noted that the 'shielding model' only holds for a S_N2 type mechanism, thus indicating that the reaction of *cis*-$NPAz_2(NPAmCl)_2$ with aziridine to $NPAz_2(NPAmAz)NPAmCl$ actually proceeds via the five-coordinated transition state.

Whereas a possible basis for the higher reactivity of the PPyrCl centre relative to PPipCl or PMorphCl can be found in the lower steric demands of the pyrrolidino group, a more satisfactory explanation for the observed sequence in reactivity is presented by the different electronic contributions of the amino substituents to the PAmCl centres. In general, the electron-donating character of Am will tend to destabilize the PCl bond in geminal position and so enhance the reactivity towards a S_N2 type substitu-



(XX) (XXIII)

Fig. 2. Schematic representation of the steric hindrance of the intermediate tetra-substituted products in reactions 6 and 9.

TABLE IV. ^1H- and ^{31}P NMR Chemical Shifts (ppm) and Coupling Constants (Hz).

Compound	$\delta(CH_2)$	$^3J_{PH}$	$\delta(PAz_2)$	δ(PAmCl)	δ(PAmAz)	$\delta(PAm_2)$	$^2J_{PP}$
XII	2.15	16.0	36.5	32.0			32.0
	2.07	16.0					
XIII	2.08	17.0	36.7		27.3		33.5
	2.04	17.0					
	1.97	16.0					
XIV	2.14	17.0	36.8	31.7			33.3
	2.07	17.0					
XV	2.08	16.0	36.9		28.6		32.8
	2.04	16.0					
	1.93	16.0					
XVI	2.14	16.5	36.9	31.3			33.6
	2.07	16.5					
XVII	2.08	16.5	36.9		28.4		33.2
	2.04	16.5					
	1.96	16.0					
XVIII	2.05	15.5	36.8		27.4		33.8
	1.94	15.5					
XX	2.14	17.0	36.0	27.5			30.4
XXI	2.12	17.0	36.6	27.7			31.9
XXII	2.12	17.0	36.7	27.2			31.9
XXIII	2.25	17.0	36.0	27.4			31.7
	2.09	17.0					
XXIV	2.22	17.0	36.8	28.0			32.7
	2.06	17.0					
XXV	2.24	17.0	36.9	27.7			32.4
	2.08	17.0					
XXVI				30.4		19.6	40.7
XXVII				29.5		19.0	41.6

tion. As morpholine is a weaker base than piperidine and pyrrolidine, this might indicate a less electron-donating substituent effect and hence a lower reactivity of the PAmCl centre. This is consistent with the relative ease of preparation of $(NPPip_2)_2NPPipCl$ (XXVI) and $(NPMorph_2)_2NPMorphCl$ (XXVII) (Experimental Part V), whereas $(NPPyr_2)_2NPPyrCl$ reacts further to $(NPPyr_2)_3$.

As may be seen in Table IV no large variations in the values of δ(PAmCl) are observed, indicating that we deal with rather similar compounds. Concerning the $^2J_{pp}$ values, a trend towards smaller values for the compounds containing pyrrolidino groups in the series $(NPAz_2)_2NPAmCl$, *trans*-$NPAz_2(NPAmCl)_2$ and *cis*-$NPAz_2(NPAmCl)_2$ can be distinguished. This is in agreement with the findings of Finer [19], who stated that the more electron-donating the substituents on the phosphorus atom in a P–N–P unit, the smaller the value of the $^2J_{pp}$ coupling constant. However, the increase in $^2J_{pp}$ on going from (XII) to (XIII) and from (XX) to (XVIII) does not fit this rule.

The cytotoxic activity of the prepared aziridino derivatives will be published elsewhere [20].

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